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DNA POLYMORPHISM OF INTERLEUKIN IL-4 OF NASAL MUCOSAL STEM CELLS IN NASAL POLYPS OF IRAQI PATIENTS

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ABSTRACT

Nasal polyposis (NP) consists of recurrent, multiple masses originating in the upper part of the nose &extend into the nasal cavity from the middle meatus, resulting in nasal blockages and restricted airflow to the olfactory region. Being of inflammatory origin, the polyp stroma is highly oedematous with a varying density of inflstreamry cells. NP are easily accessible for immunological and histologic. l studies and an increasing number of articles have appeared in recent years concerning the condition.

Interleukin (IL) -4is a cytokine that which is considered to play a pivotal role in the pathogenesis of asthma & some allergic conditions was chosen to be subjected to study in this paper.

DNA was extracted from NP and used polymerase chain reaction (PCR) & compared to the DNA of intact mucosa concerning IL-4 polymorphism.

The aim of this study is: to clarify the role of IL- 4 polymorphism by using PCR technology in nasal mucosal stem cells in Nasal Polyps of Iraqi patients and use it as a biomarker.

KEYWORDS: Nasal Mucosal Stem Cells, Nasal Polyps & Interleukin-4

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Original Article

INTRODUCTION

Nasal polyps are tissue masses in the nose that originate from the mucosa of paranasal sinuses, frequently the anterior ethmoid complex. They are considered to result from chronic inflammation, but the initial stimulus for the inflammation is not known. **[**Andrews AE *et al*., 2005**].**

Lesions are round, soft, semi translucent, pale and glistening that could descend between the lateral nasal wall and the middle turbinate into the nasal cavity causing symptoms such as nasal obstruction, rhinorrhea, nasal congestion, facial pressure and hyposmia **[**Fokkens WJ *et al*., 2005**].**

Interleukins are group of cytokines (signaling molecules), primary expressed from white blood cells **[**Brocker C *et al*., 2010**].**

The function of the immune system depends mostly on interleukins, and any deficiency of one of them could cause immune deficiency or autoimmune diseases. The greater part of interleukins are synthesized by helper CD4 T lymphocytes, in addition to monocytes, macrophages, and endothelial cells, they support the differentiation of T and B lymphocytes **[**Ofra BM *et al*., 2010**].**

Interleukins IL-4, IL-3, IL-1 as well as tumour necrosis factor (TNF) have been expressed in the polyps, and, in various combinations, may have synergistic effects on VCAM-1 expression. Another adhesion molecule,

P-selectin, also probably has a role in the initial adhesion of eselectionls to the polyp endothelium[Braddinget *al.*,2016& Mygind *et al*.,2000].

PATIENTS AND METHODS

Sample Collection

The samples of this study were (58) Patients were selected who undergo of nasal surgery in Gazi Al-Hariri Teaching Hospital, Baghdad, Iraq from May 2013 to January 2014. They were as groups:

- **Control Group:** (22) Samples of mucosa of inferior turbinate were obtained from patients undergo septorhinoplasty intended for septal deviation (The inferior turbinate mucosa was grossly normal, with no evidence of infection or inflammation), they were used as normal controls.
- **Study Group:** (36) Samples of nasal polyposis were obtained from patients who had undergone nasal polypectomy (Table 1).

All patients were in the third decade of life except 1 NP case was 10 years old.

Table 1: The Study Sample

Group	Females		Males		Total Number
	Number	%	Number	%	
Control	6	27.3	16	72.7	22
Study	22	61	14	39	36

Extraction of DNA

DNA extraction was, according to Geneaid kit (USA). The samples were small sections which sliced (up to 25 µg) from the blocks of paraffin-embedded tissue and transferred to a 1.5 ml Microcentrifuge tube. 1 ml of absolute ethanol was added to wash the sample pellet, and then DNA was purified.

Measurement of the concentration and purity of DNA by using the Nanodrop was done. The concentration was (1.84-1.98) mg/ml.

Agarose Gel Electrophoresis

The DNA, which extraction was confirmed by agarose gel electrophoresis [Sambrook J, et al., 1989], and visualized at UV- transmillator, then Image for the gale was captured.

Primers of IL-4

Primer sequence for IL-4 was

Forward	**F5`-GAA CAG CCT CAC AGAG CAG.3`**
Reverse	**R5`-AGC ACA GTC GCA GCC.3`**

PCR (Polymerase Chain Reaction)

PCR was according to kit (AccuPower® ProFi Taq PCR PreMix). In 30 cycles the denaturation was 94 °C for 15-20 sec, annealing was 60°C for 45 sec, extension was 72 °C for 1min and final extension was 72 °C for 10 sec.

(5 µl) of the reaction mixture was loaded directly on agarose gel without adding a loading dye to analyze the PCR products.

RESULTS

IL-4 showed positive results in the NP group by using PCR, while all control samples were negative for IL-4. Pearson Chi square showed a significant relation of PCR results as compared to pathological groups. (Table 2& figure 1).

Table 2: Study Groups

PCR Results	Groups				Total Number
	Control		Nasal Polyp		
	Males	Females	Males	Females	
Positive	**0**	**0**	**6**	**15**	**21**
Negative	**6**	**16**	**8**	**7**	**37**
Total	**6**	**16**	**14**	**22**	**58**
P value	**0.03**				

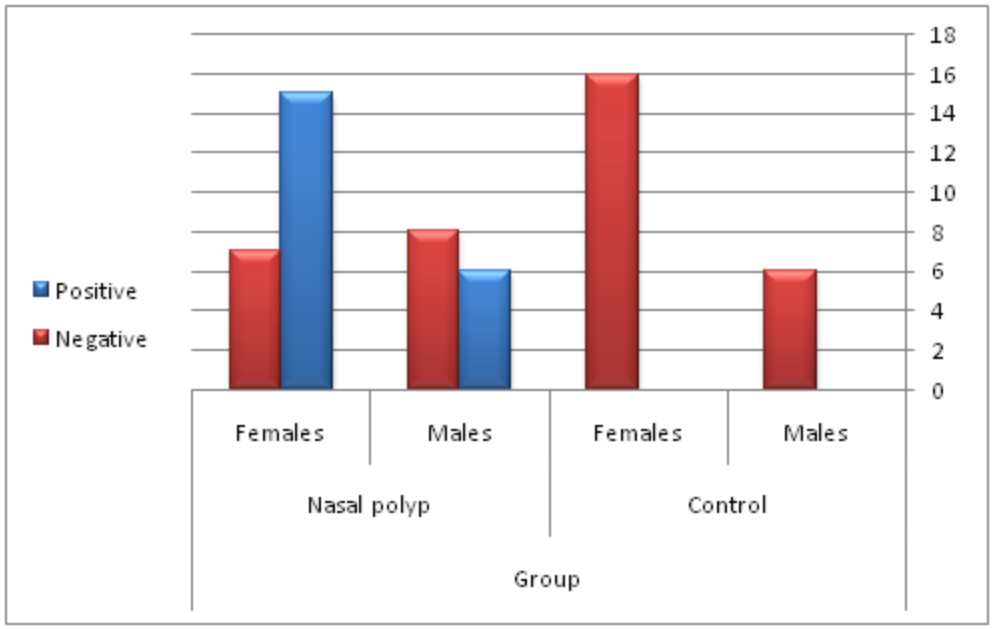

Figure 1: PCR Results with Sample Groups

The DNA electrophoresis results were shown in the figure 2. In lane1 DNA marker (Lambda DNA\ EcoR+ Hind 111) which had 4bands(21.226,5.148,4.268,2.027bp), (22) years old female with nasal polyp had one band(21.226bp) compare with DNA marker, (21) years old male with nasal polyp had one faint band (21.226bp) compare with DNA marker, 19 years old female with nasal polyp had one band(21.226bp) compare with DNA marker, (23) years old female with nasal polyp had one band(21.226bp) compare with DNA marker, (20) years old female with nasal polyp had one band(21.226bp) compare with DNA marker, (22) years old male with nasal polyp had one band(21.226bp) compare with DNA marker, (26) years old male with nasal polyp had one band(21.226bp) compare with DNA marker.

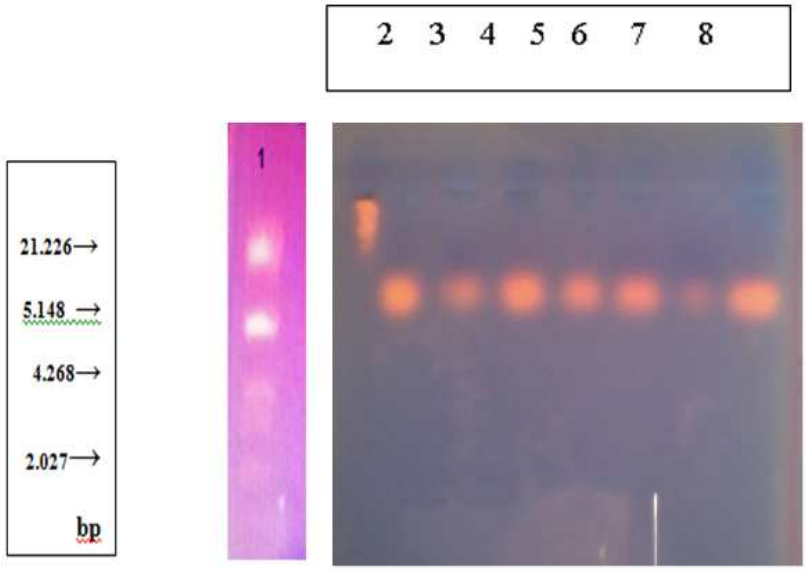

Figure 2: The Pattern of Agarose Gel Electrophoresis of IL-4

- **Lane- 1:** DNA marker (Lambda DNA\EcoR+Hind 111)
- **Lane-2:** (22) years old female with nasal polyp
- **Lane-3:** (21) years old male with nasal polyp
- **Lane-4:** (19) years old female with nasal polyp
- **Lane-5:** (23) years old female with nasal polyp
- **Lane-6:** (20) years old female with nasal polyp
- **Lane-7:** (22) years old male with nasal polyp
- **Lane-8:** (26) years old male with nasal polyp.

DISCUSSIONS

All patients were considers as non-allergic, non-asthmatic depends on medical history, the results shown significant increase of IL-4 expression in nasal polyps compared with control group.

IL-4 is thought to produce a messenger RNA blocking the production of other cytokines such as IFN-y, participating in the mechanism of polyp formation in cystic fibrosis[Nues et.al 2010].

The closely related IL-4 and IL-13, share biological functions that are considered important in the development of airway inflammation, including induction of the IgE isotype switch, increased expression of VCAM-1, eosinophil transmigration across the endothelium stimulation of mucus production and Th2 cell differentiation, leading to release of IL-4, IL-5, IL-9, IL-13 and eotaxin. Furthermore[Bachert et al., 2005].

Histologically, nasal polyps can be divided into 4 types: edematous (eosinophilic), fibrotic (non eosinophilic), glandular, and atypical [Davidsson A& Hellquist HB,.1993;Hellquist HB.,1996 ;Hellquist HB,.1997; Ferreira Couto LG *et al*.,2008].

The types of inflammatory cells that infiltrate nasal polyps differ in Asian and Western patients [Bernstein JM *et al*., 1997**]**. Bachert C et al, reported that eosinophilic nasal polyps were found in more than 80% of Western patients with NP[Bachert C *et al*.,1997], whereas Hao et al, reported that the incidence of fibrotic polyps (mainly accumulation of lymphocytes and neutrophils) was relatively higher (more than 40%) in Asian patients**[**Hao J *et al*.,2006**]**.

Zhang et al, reported a lower incidence of eosinophilic nasal polyps in Chinese patients than in European patients. Also found that noneosinophilic polyps were present in nearly 60% of Chinese patients with NP, while eosinophilic polyps were present in less than 40% of those patients. So, the pathogenic mechanisms of nasal polyps vary in Eastern and Western populations**[**Zhang N *et al*.,2006**]**.

As conclusion the IL-4 has a role in detection nasal mucosal stem cells in Nasal Polyps by using PCR technology (IL- 4 polymorphism) of Iraqi patients and can used it as a biomarker.

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